Exhibit C

NOTEBOOK NO. 2369

ISSUED TO Randy Saiki

ON _____ 19__

DEPARTMENT Human Genetics

RETURNED _____ 19__

SCIENTIFIC NOTEBOOK CO.
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[redacted]

From Page No. X

Rec'd 80μl Taq polymerase from David Gelfand. Tube is labeled "fraction VIII A", will call it lot 3B. (Shirley and David have 3A, more concentrated.) This stuff is at 10 u/μl using their activated salmon sperm (or is it calf thymus) DNA assay. Titrate for PCR amplification.

A, F:	1μl per 100μl rxn.		
B, G:	1/2 "	A-E:	Molt4
C, H:	1/4 "	F-J:	GM2064
D, I:	1/8 "		
E, J:	1/16 "		

Molt4 and GM2064 @ 100 ng/μl
PC03 and PC04 @ 10μM, dNTP @ 10mM each

35μl	Molt4 or GM2064
35μl	10x Taq salts
35μl	PC03
35μl	PC04
35μl	DMSO
52.5μl	dNTP
122.5μl	H_2O
350μl →	5', 95°

Cool to RT and divide into one 100μl ~~volume~~ sample and four 50μl samples (50μl left over). Add 1μl Taq polymerase (lot 3B, 10 u/μl) to the 100μl volume and mix. Prepare four 50μl serial dilutions in four remaining samples. two-fold

Final concentrations of enzyme per 100μl reaction volume: 1μl, 1/2, 1/4, 1/8, 1/16.

Overlay with mineral oil

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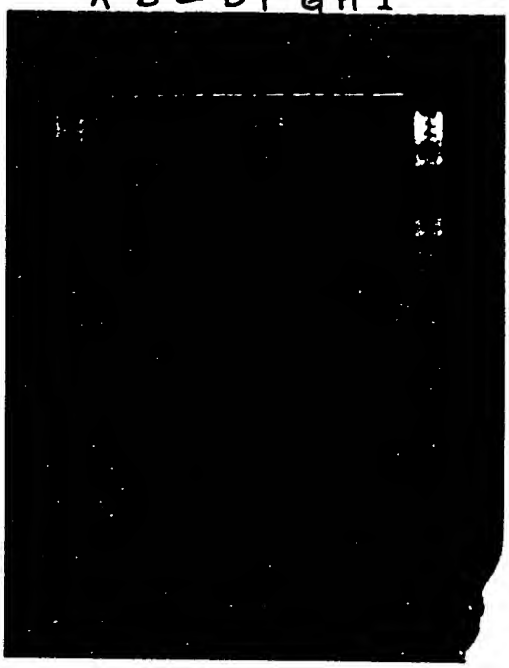
Save remaining 50µl (1/16 dil'n) and store @ 4° (just in case enzyme works at < 1/16 µl per 100 µl).

Subject to 24 cycles: 2 min ramp, 35° to 95°
2 min ramp, 95° to 35°

After last cycle, incubate additional 5 min at 65° to complete final (25th) extension.

Extract oil with $CHCl_3$.

Load 5µl each A-D and F-I on 4% NuSieve / 0.5% agarose / 1xTBE.



~~[illegible]~~

Got PCR product in Molt4 at all four dilutions, even 1/8 µl! As expected, nothing in GM2064.

Background is very low, virtually nonexistent for 1/4 and 1/8 µl samples. Maybe combination of enzyme and "fast ramp" protocol is responsible.

Need to check 1/16 µl samples; might be band there, too.

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Compare titrations of lot 2A and lot 3B of Taq polymerase using "Pro/Petle" protocol.

A	I :	1 µl	E M :	1/16 µl	
B	J :	1/2	F N :	1/32	A-H: lot 2A
C	K :	1/4	G O :	1/64	I-P: lot 3B
D	L :	1/8	H P :	1/128	

50µl	Molt4
50µl	10x salts
50µl	PC03
50µl	PC04
50µl	DMSO
75µl	dNTP
175µl	H_2O
500µl →	10', 95°

Prepare eight 50µl two-fold serial dilutions with 1µl lot 2A or lot 3B as described on page 88.

Subject to 24 cycles in ProPetto: 2½' min ramp, 37 to 95°
3' min ramp, 95 to 37°

After last cycle incubate 5' @ 60° to complete 25th cycle extension.

Load 5µl each onto 4% NuSieve/0.5% agarose/1x TBE.

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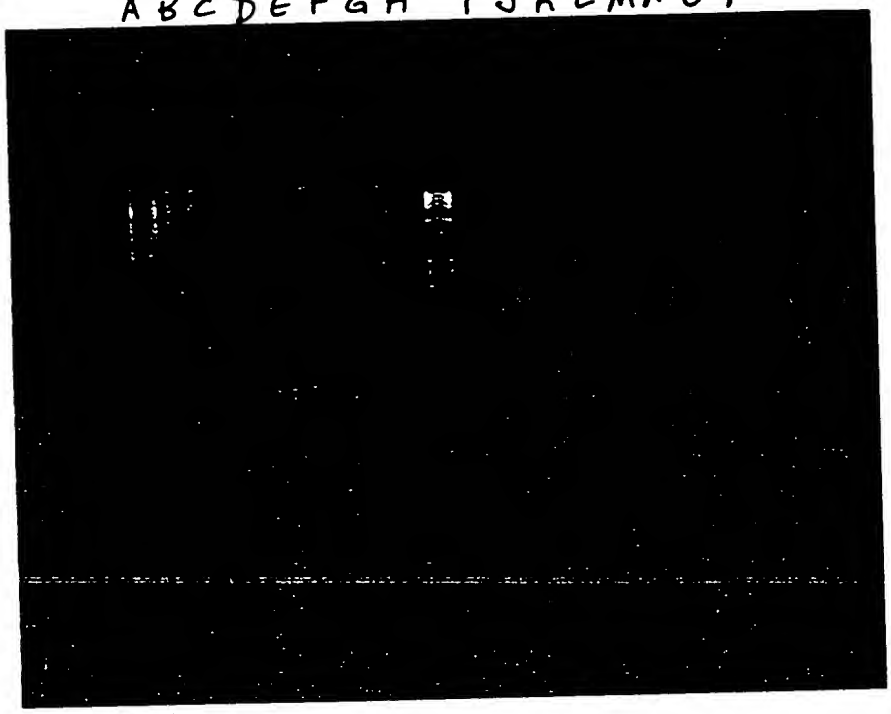
A B C D E F G H I J K L M N O P



amplification

Don't see a cut-off in lot 2A anymore. Can see a band as far down as 1/64 µl (lane G). Also, best S/N ratio ratio is at 1/16 µl instead of 1/8 µl.

Lot 3B seems to peak at 1/2 µl although this should be rechecked. [illegible]

There seems to be more background in these samples than in those done "fast ramp". Need to compare on same gel to be sure.

This gel show effect of polymerase on S/N ratio quite nicely.

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Lot 3 Taq polymerase seems to be losing activity. Initially, optimal concentration (for PC03/04) was at 2.5 u per 100 µl reaction. Over the last 2-3 weeks activity has gradually ~~disappear~~ dissapated. Most recent attempts have failed (not recorded). Only a very weak PCR ~~products~~ product was seen with 5u. Russ, Dory, and Steve have had similar experiences.

Unlike lot 2A, the storage buffer for lot 3 does not contain the non-ionic detergents Tween 20 or NP40.* David Gelfand's experience with this polymerase indicates that it is a sticky enzyme and he routinely uses both detergents during purification to improve yield and during assay to stimulate activity.

May be that in the absence of detergents and at -20° the enzyme is aggregating. Addition of "soap" to either the storage buffer or the PCR reaction may restore activity. Will try the latter first.

	u/100µl
A H:	1/2
B I:	1/4
C J:	1/8
D K:	1/16
E L:	1/32
F M:	1/64
G N:	1/128

A-G: (-) detergent
H-N: (+) detergent (0.05% each)

*Another difference is that lot 3 contains 200 ug/ml gelatin. Lot 2 doesn't.

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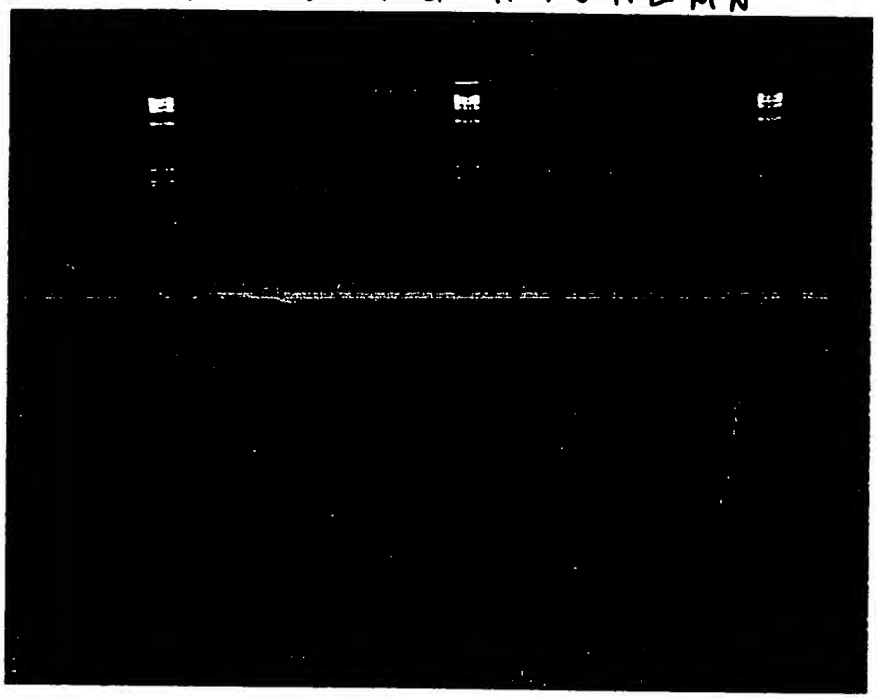
Load 5µl each onto 4% NuSieve / 0.5% agarose / 1x TBE
→ 100v, 90'

A B C D E F G H I J K L M N



Detergents definitely have some effect. Without them can only see a band in ~~AAAAAA~~ 5u sample (A). But with them, can see bands in 5u (H), 2.5 (I), and very faintly in 1.25 (K).

Although Tween and NP40 helped, activity still is not as good as it was originally (p. 81).

Maybe that adding more detergents or adding instead to the storage buffer will ~~work~~ be better.

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Molt4 @ 100 mg/ml, PC03 and PC04 @ [redacted] 10μM
dNTP @ 40mM (p.99), Tween/NP40 @ 0.5% each

A-G:		H-N:	
50μl	Molt4	50μl	Molt4
50μl	10x salts	50μl	10x
50μl	PC03	50μl	PC03
50μl	PC04	50μl	PC04
50μl	DMSO	50μl	DMSO
75μl	dNTP	75μl	dNTP
175μl	H_2O	50μl	Tween/NP40
500μl → 10', 95°		125μl	H_2O
		500μl → 10', 95°	

Dispense each 500μl mix into one 100μl sample and six 50μl samples. Add 1/2μl lot 3 to the 100μl sample and ~~dilute~~ serially dilute, preparing seven 50μl two-fold serial dilutions: 1/2μl to 1/128μl.

Overlay with mineral oil and subject to 24 cycles on Sninsky's ProPette: 3' ramp 37° to 95°
3' ramp 95° to 37° (hot water set at 102°)

After last cycle, incubate additional 10' at 56°.

Extract oil with $CHCl_3$. (Samples H-N ~~became~~ became cloudy. Probably interaction of detergents with chloroform.)

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Follow up on expt. described page 101 by ~~determining~~ seeing if adding detergents directly to enzyme stock is a better way to go. Gelfand suggests 0.5% each is a good starting point.

19 µl	Taq polymerase (lot 3, 10 u/µl)
1 µl	10% Tween 20 / 10% NP-40
20 µl	Taq pol, 9.5 u/µl, 0.5% each detergent → incubate @ RT for ~10', mixing throughly. Store @ 4°

A E: 1
B F: ½
C G: 1/4
D H: 1/8
} µl Taq

A-D: Taq w/o detergent
E-H: Taq w/ detergent

reagents as desc. page 101

30 µl	Molt4
30 µl	10x salts
30 µl	PC03
30 µl	PC04
30 µl	DMSO
45 µl	dNTP
105 µl	H_2O
300 µl → 10', 95°	

Prepare ~~for~~ two 300 µl mixes. Divide each into one 100 µl sample and three 50 µl samples. Add 1.0 µl enzyme, with or without detergent, to the 100 µl sample and serially dilute 50 µl into the three 50 µl samples.

Amplify and workup as described p102 except use our Pro/Petter with this program: 2½ ramp, 35° to 95°
3' ramp, 95° to 35°

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Tag PCR: (cont'd)

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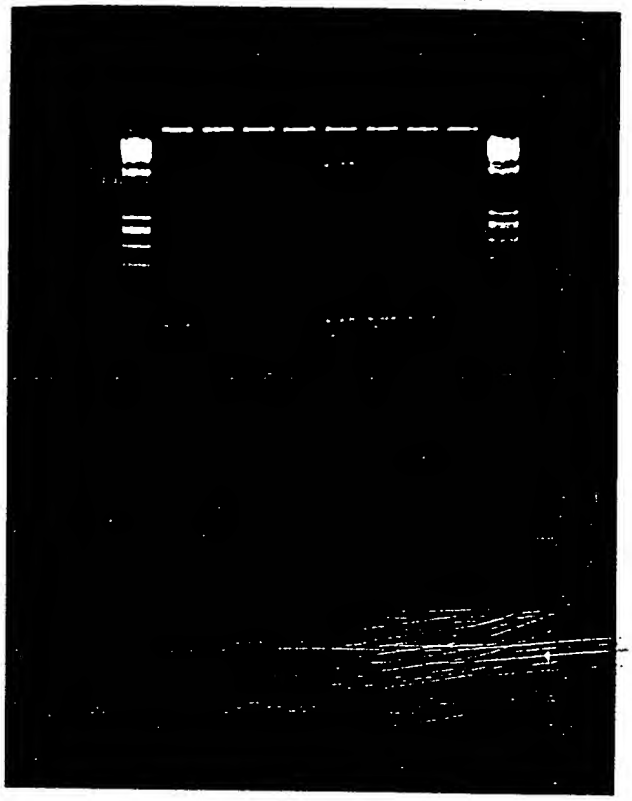
Load 5µl each sample onto 4% NuSieve/0.5% agarose/1x TBE.
→ 100 v

A B C D E F G H



This is it! Activity of enzyme with detergent is as good as (maybe even better) than original titration (see p. 81).

Based on this expt. best conc. of enzyme is either 5.0 u (F) or 2.5 u (G). Former may have a teeny bit more PCR product, but latter has less background. (Either is fuckin' good.)

Looks as if activity in (-) detergent enzyme has gotten even worse. Can barely see the ~~1µl~~ 1/2 µl sample (B).

Should add detergent to the ~~remaining~~ remaining enzyme stock.

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